Received Date : 07-Sep-2015

Revised Date : 24-Feb-2016

Accepted Date : 29-Feb-2016

Article type : Original Article

Ex vivo sentinel lymph node mapping in colorectal cancer using a magnetic nanoparticle tracer to improve staging accuracy: a pilot study

Joost J Pouw MSc, PhD[1*], Maarten R Grootendorst MSc[1,2,‡], Joost M Klaase MD, PhD[2], Joop van Baarlen MD[3], Bennie ten Haken PhD[1,*]

MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, P.O. Box 217, 7500AE, Enschede, The Netherlands. [1]

Department of Surgery, Medisch Spectrum Twente, P.O. Box 50.000, 7500KA, Enschede, The Netherlands. [2]

Laboratorium Pathologie Oost Nederland, P.O. Box 516, 7550AM, Hengelo, The Netherlands. [3]

**Authors for correspondence: Email: J.J.Pouw@utwente.nl; B.tenHaken@utwente.nl Tel: +31 (0)53 489 2158*

‡Current affiliation: Department of Research Oncology, King's College London, Great Maze Pond, SE1 9RT, London, UK.

Conflicts of interest: None

ABSTRACT

Aim: Nodal status is the most important prognostic factor in colorectal cancer (CRC). Small occult metastases may remain undetected on conventional histopathological examination, potentially resulting in undertreatment. E*x vivo* sentinel lymph node mapping (SLNM) can be used to improve nodal staging accuracy, but currently used tracers suffer from drawbacks, which hampers implementation of the technique in routine clinical practice. Magnetic tracers have an optimal size for sentinel lymph node (SLN) retention and allow objective quantitative selection of SLNs and therefore have great potential for SLNM in CRC. The study evaluates the feasibility of *ex vivo* magnetic SLNM and compares the performance of this technique to blue dye SLNM.

Method: Twenty-eight *ex vivo* SLNM procedures were performed in 27 histological node-negative patients with CRC using a magnetic tracer and blue dye. A magnetometer was used to select magnetic SLNs after formalin fixation of the CRC specimen. Both magnetic and blue SLNs were subjected to serial sectioning and immunohistochemical staining to reveal occult metastases.

Results: At least one SLN was successfully identified in 27/28 (96%) and 25/28 (89%) of the cases with the magnetic technique and blue dye. Isolated tumour cells were detected in 10 patients. This was predicted with 100% sensitivity and accuracy using the magnetic technique, and with 91% sensitivity and 96% accuracy using the blue dye technique.

Conclusion: This study demonstrates that *ex vivo* magnetic SLNM is a feasible technique for use in routine clinical practice, which improves nodal staging accuracy of CRC patients.

What does this paper add to the literature?

The study is the first to use a magnetic tracer and handheld magnetometer for SLNM in colorectal cancer. The procedure improves the accuracy of nodal staging by detecting small occult metastases, which potentially allows improved treatment. The *ex-vivo* procedure can easily be implemented in routine clinical practice.

Keywords*: Superparamagnetic iron oxide; sentinel lymph node mapping; magnetic tracer; colorectal cancer; staging*

INTRODUCTION

Over 1.3 million patients are annually diagnosed with colorectal cancer (CRC). In the more developed regions of the world, CRC is the second most common cause of cancer related death, accounting for over 300.000 deaths in 2012 [1]. Treatment with curative intent consists of *en-bloc* resection of the affected colorectal segment and adjacent lymphatic drainage. Current guidelines demand that at least 12 lymph nodes (LNs) should be retrieved from the specimen and examined with haematoxylin & eosin (H&E) staining of a single cut surface for the presence of metastasis [2]. The presence of nodal metastasis is the most important prognostic factor, and determines whether adjuvant chemotherapy is indicated. Up to 30% of the node negative patients, however, develop recurrent disease within five years of surgery with curative intent [3, 4]. Several retrospective studies attribute this high recurrence rate to the presence of occult nodal involvement, which remains undetected on conventional histopathological analysis [5-7]. Patients suffering from occult nodal involvement may therefore be understaged, potentially leading to under-treatment [6-8]. Although these small micrometastases

(0.2-2 mm) and isolated tumour cells (ITCs, < 0.2 mm) can be detected using so called 'ultrastaging' (serial sectioning and immunohistochemical staining or molecular pathology techniques), this is too expensive and time consuming to perform on the large number of LNs retrieved from the colorectal specimen in routine clinical practice. Since ultrastaging is not performed routinely, occult nodal involvement is missed. This results in inadequate staging, and potentially inadequate treatment of a large group of patients. The goal of the present study is to provide a method to improve the accuracy of staging of conventional node negative CRC patients that can easily be implemented in routine clinical practice.

Sentinel lymph node mapping (SLNM) allows identification of lymph nodes that receive direct lymphatic drainage from the tumour area; the so-called sentinel lymph nodes (SLNs). Since occult nodal involvement is predominately found in SLNs [5], their status is representative of the entire colorectal nodal basin. By subjecting only the SLNs to ultrastaging, the presence of occult metastasis can be accurately detected, without considerably increasing cost and labour, thus allowing improved nodal staging.

At present, SLNM is performed by injecting a blue dye and/or radioisotope tracer peritumorally, followed by visual and/or gamma probe detection of discolored and/or radioactive SLNs. The procedure can be performed *in vivo* and *ex vivo*, with a similar detection rate and accuracy [9, 10]. Aberrant lymphatic drainage is reported in only approximately 1-10% of cases and therefore a limited drawback when an *ex vivo* approach is used for SLNM. An important advantage of the *ex vivo* approach is that the patient is not exposed to the tracer and therefore not at risk of developing an allergic reaction [11, 12]. Furthermore, an *ex vivo* procedure does not add time to the surgery. Although the colloidal properties of radiotracers are beneficial for SLN detection [13, 14] they are generally not used in an *ex vivo* setting because the use of radioisotopes is

strictly regulated [15]. Blue dye is readily available and not limited by radiation safety legislation, but, due to the small size of the blue dye particles, they rapidly distribute through the lymphatic system, not only colouring the true SLNs but also higher echelon nodes. In routine clinical practice, all LNs are dissected from the formalin fixed tissue by the pathologist usually on the day after surgery. Because of the distribution to higher echelon nodes and dilution of the blue dye in formalin, SLN identification must be performed shortly after injection of the tracer. Widespread clinical implementation of SLNM in CRC patients requires a tracer that allows SLN detection after formalin fixation during routine pathological analysis, without regulatory and logistical barriers that hinder its day-to-day use.

Magnetic tracers have several advantageous properties for use in SLNM in CRC. We previously demonstrated that they accumulate in the lymph nodes in colorectal cancer after *ex vivo* administration using non-clinical laboratory equipment [16]. Recently the use of magnetic tracers was also evaluated for SLN biopsy in breast cancer patients [17]. These consist of superparamagnetic iron oxide (SPIO) nanoparticles with a hydrodynamic size of 50-150 nm, which is favourable for retention in the SLN [18]. The colloidal properties of the tracer also prevent dilution from formalin fixation. Another advantage is that the presence of a magnetic tracer in LNs can be easily quantified with a handheld magnetometer [18], allowing objective discrimination between SLNs and higher echelon nodes. In addition, magnetic tracers do not suffer from regulatory and logistical barriers associated with radioactivity, thus providing flexibility to perform SLNM. Because of these advantageous properties magnetic SLNM has great potential to improve nodal staging accuracy in CRC patients in routine clinical practice. In this study we evaluate the feasibility of using *ex vivo* magnetic SLNM in a routine clinical work flow

and compare this novel technique with *ex vivo* blue dye SLNM with SLN identification after formalin fixation.

METHOD

Patients with clinical stage I/II CRC scheduled for curative surgery were eligible for inclusion. The study was performed in agreement with the local ethics committee and all patients gave written informed consent. Exclusion criteria included the presence of distant metastasis, intraoperative gross nodal involvement and preoperative neoadjuvant chemoradiotherapy. Forty *ex vivo* SN procedures were performed in 39 patients including one patient who had two tumours

resected. All patients underwent a standard oncological resection and *en-bloc* lymphadenectomy according to the location of the tumour. The presence of nodal metastasis was detected by conventional histological analysis in 12 of the 39 patients. Since the goal of the study was to improve the accuracy of staging of the patients without nodal metastasis on conventional histological analysis, the 12 patients were excluded from further study. The remaining 27 node negative patients (28 procedures) comprise the group investigated in the study.

Sentinel lymph node mapping procedure

Immediately after resection, the fresh surgical specimens were sent to the pathology department. Colonic specimens were opened antimesentrically by the pathologist or technician, and rectal specimens were opened on the anterior border opposite of the mesorectum, leaving the mesorectum intact. The tumour was located, and the diameter determined.

First, 2 mL of magnetic tracer were injected submucosally at four sites around the tumour. In the first 10 procedures an MRI contrast agent, Endorem® (Guerbet B.V., Gorinchem, The Netherlands), was used as magnetic tracer. In the remaining 18 procedures, a magnetic tracer optimized for SLN localization [18], Sienna+® (Endomagnetics Ltd, Cambridge, UK), was used. Endorem® consists of SPIO nanoparticles with a mean hydrodynamic diameter of 111 nm and contains 11.2 mg iron (Fe)/mL. Sienna+® has a concentration of 28 mg Fe/mL and the nanoparticles have a mean hydrodynamic diameter of 59 nm. The original tracer was changed because a new tracer specifically designed for SLN localization became commercially available during the course of the study. Since the production of the MRI contrast agent was discontinued, it was used as an alternative. A gentle massage of the injection sites was performed for three minutes to promote distribution of the *ex vivo* tracer. Subsequently, an optimized dose of 0.5 - 2 mL Patent Blue V (Guerbet B.V., Gorinchem, The Netherlands) was injected at the same sites (0.5 mL/cm of tumour diameter [19]), followed by a massage of five minutes. The magnetic tracer was injected first, because a more difficult distribution of the viscous magnetic tracer was anticipated than with the more fluidly blue dye.

After administration of the tracers the specimen was fixated in formalin (10%) for 24-72 hours. Following fixation, all LNs were harvested from the specimens according to the standard histopathological protocol. All harvested LNs were individually placed on the probe of a handheld magnetometer, SentiMAG® (Endomagnetics Ltd., Cambridge, UK), to select the LNs with uptake of magnetic tracer (Fig. 1). The probe was balanced in air (away from any magnetic material) before each measurement to compensate for any drift of the signal. The signal is proportional to the amount of magnetic tracer [18, 20] and was used for the quantification of tracer uptake. An SLN was defined as a lymph

node with uptake of magnetic tracer. If more than three nodes with tracer uptake were identified, the three nodes with the highest SPIO uptake were designated as SLNs. Blue nodes were also defined as SLNs, if more than three blue nodes were detected and the first three blue nodes closest to the tumour were selected as SLNs. This definition is similar to the definition used by Faerden et al [21]. A procedure was considered successful if at least one SLN was identified. If ITCs and/or micrometastasis was found in a LN, but not in one of the SLNs, this was considered to be a false negative result.

Histopathological analysis

All lymph nodes were embedded in paraffin and stained with haematoxylin and eosin (H&E). In addition to this conventional staging, SLNs of the node negative patients were subjected to ultrastaging. The SLN was additionally sectioned at 250 μm intervals, and coloured with Cam5.2, an anti-body against keratin, and H&E. Positive cells were categorised as isolated tumour cells (<0.2 mm) or micrometastasis (0.2 - 2 mm). Rare single positive staining cells with immunohistochemical staining lacking cytological characteristics of malignancy were considered negative. In the first ten procedures ultrastaging was performed on all resected nodes, to rule out false negative results. If none was detected ultrastaging of the non-SLNs would be omitted in the remaining procedures. Ultrastaging of the non-SLNs was not performed in the vast majority (~83%) of studies reported in literature [5], and omission was therefore justified.

Statistical analysis

Means and range were calculated for continuous variables. The number of SLNs identified with both techniques we compared using a paired *t* test. Statistical analyses were performed using SPSS software version 22.0 (SPSS Inc., Chicago, IL), and $P < 0.05$ was considered significant.

RESULTS

Twenty-eight *ex vivo* SLNM procedures were performed in 27 patients who underwent intended curative surgery for colorectal cancer and were lymph node negative on routine histological staining. Patient and tumour characteristics are provided in Table 1. At least one SLN was successfully identified in 27/28 procedures with the magnetic tracer, and in 25/28 procedures with the blue dye, resulting in an identification rate of 96% for the magnetic technique and 89% for the blue dye technique. The results of the SLNM procedure are given in Table 2.

A total of 607 LNs were dissected, of which 199 (mean 7.4, range 1-16) were magnetic, and 205 (mean 8.2, range 3-36) were blue. More than three LNs with tracer uptake (blue and/or magnetic) were found in all but three procedures. The uptake of magnetic tracer in the LNs could be easily quantified with the handheld magnetometer, and allowed an objective selection of three SLNs with the highest uptake in all procedures. From all LNs with magnetic tracer uptake, the three with the highest uptake per patient were designated to be magnetic SLNs: the LNs with lower uptake were considered to be non-SLNs. Of the blue lymph nodes the three closest to the tumour were designated to be blue SLNs, the other blue LNs located further away were considered to be non-SLNs. It is therefore possible that a node might demonstrate tracer uptake (blue and/or magnetic), but would not have been considered to be a SLN. A total of 120 SLNs were selected from all nodes with tracer uptake (blue and/or magnetic).

Of the 120 SLNs, 33 were selected as both magnetic and blue SLNs, 46 were selected as magnetic SLN only and 41 were selected as blue SLN only (Fig. 2). Only 11 magnetic SLNs were not coloured blue and only 11 blue SLNs were not magnetic.

Ultrastaging of the SLNs by serial sectioning and immunohistochemical staining showed the presence of ITCs in 10/27 conventional node negative patients. No micrometastases were detected. On a node-to-node basis, ITCs were detected in 11 both magnetic and blue SLNs, six solely blue SLNs and five solely magnetic SLNs. Isolated tumour cells were found in two non-SLNs of one patient. On a patient-to-patient basis the magnetic technique accurately predicted the presence of occult tumour cells in all patients, but in one patient ITCs were found solely in a magnetic SLN that was not blue, resulting in a false negative result with the blue dye. No false-negative results were identified when ultrastaging all the non-SLNs of the first ten patients, therefore ultrastaging of the non-SLNs was omitted in the remaining procedures of this pilot study.

DISCUSSION

The presence of nodal metastasis is the most important prognostic factor in CRC and is used to determine whether a patient should receive adjuvant treatment. Hence, accurate nodal staging is of great importance. SLNM can be used to improve the accuracy of nodal staging by identifying the nodes at risk of harbouring occult metastasis, and subjecting these nodes to ultrastaging. This study demonstrates the feasibility of a magnetic technique for *ex vivo* SLNM to improve the accuracy of nodal staging in routine clinical practice. The use of a magnetic nanoparticle tracer and handheld magnetometer was recently evaluated for *in vivo* SLNB in breast cancer patients and shown not to be inferior to the standard technique [17]. Our group previously explored the potential of using magnetic nanoparticles for *ex vivo* SLNM in colorectal cancer [16, 22]. To our knowledge, the current study is the first to evaluate the magnetic technique for *ex vivo* SLNM in colorectal cancer in a routine clinical workflow. An identification rate of 96% was achieved with the magnetic tracer, which is

in line with the previously reported average identification rate of 94% with blue dye and/or radioisotopes [15, 23].

The identification rate using the magnetic technique was higher than with the blue dye. There are two possible explanations for this difference. First, the magnetometer is able to detect very low amounts of tracer, whereas small amounts of blue dye are difficult to distinguish visually. Secondly, formalin fixation of the tissue can dilute the blue dye and thus lower the concentration so that it is no longer visible resulting in a false negative. In contrast, the colloidal properties of the magnetic tracer prevent dilution, once it is taken up by the LNs. Therefore, the magnetic technique does not suffer from dilution of the tracer, allowing identification of the SLN through the presence of small amounts of magnetic tracer, even after formalin fixation.

Sentinel lymph node identification with blue dye is generally performed immediately after surgery [15] to prevent washout of the dye after formalin fixation. This procedure does not, however, fit easily into a routine clinical workflow. Identification after formalin fixation, as described by Faerden et al [21] and Smith et al [24] allows the procedure to be performed in routine clinical practice. Since the goal of this study was to develop a method for SLN identification in routine clinical practice, this approach was used for both the blue dye and magnetic tracer.

Although the magnetic tracer consists of larger particles, there was no significant difference in the mean number of blue nodes and mean number of nodes with magnetic tracer uptake. This is also most likely because of the high sensitivity of the magnetometer for small amounts of magnetic tracer, as it could result in detection of higher echelon nodes with very limited tracer uptake. Most non-SLNs with uptake of magnetic tracer (77/120 non-SLNs) displayed a signal less than 10% of that of the most

magnetic SLN, supporting the assumption that higher echelon nodes containing small amounts of magnetic tracer uptake were also detected. This results in a high number of magnetic lymph nodes. In our study, the three most magnetic LNs were designated as SLNs, and the 10% criterion was not used to discriminate between SLNs and non-SLNs. Future studies should determine whether the 10% criterion can be used further to reduce the number of magnetic SLNs. A unique property of the magnetic technique is that the quantitative signal allows the objective selection of the SLNs. Furthermore, unlike the blue dye, a standardized dose of 2 mL of SPIO was used whatever the tumour diameter. Dose optimization of the magnetic tracer is likely to reduce the number of magnetic nodes as well, as it will restrict tracer distribution to higher echelon nodes.

A large discordance between the nodes selected as SLN was observed between the magnetic and blue technique. Of the 120 SLNs, 33 were selected as both magnetic and blue SLNs, 46 were selected as magnetic SLN only and 41 were selected as blue SLN only. However, only a small proportion of magnetic SLNs (11/46) were not coloured blue and a similar small proportion of blue SLNs were not magnetic (11/41). The observed discordance is therefore likely to arise from differences in the definitions used for the selection of the magnetic and blue SLNs, the former based on an objective functional definition the latter on a subjective anatomical definition. In some procedures it was difficult to determine which lymph nodes were closest to the tumour, emphasizing the subjective nature of this definition.

Ultrastaging of the SLNs revealed ITCs in 10/27 patients (37%), which is concordant with previous studies [5]. The presence of occult metastasis was accurately predicted by the magnetic technique in all 10 patients, and in 9 out of 10 by the blue dye technique. Ultrastaging did not show any micrometastasis. The true false negative rate cannot be determined because ultrastaging of the non-SLNs was only performed in the first ten

patients, but because no false negative results were obtained in these patients, and ultrastaging was generally limited to the SLNs only [5] our results indicate that the magnetic technique accurately predicts the presence of occult metastasis. Ultrastaging of the non-SLNs was limited to the patients in whom Endorem® was used as tracer. Since a different tracer was used in the remaining patients, this could have influenced the results. The used tracers are however very similar in terms of constituency, and particle size distributions [18], and this is therefore unlikely. To date no prospective trials have been performed to determine whether patients with ITCs/micrometastasis would benefit from adjuvant treatment, but a recent meta-analysis concluded that patients with micrometastasis have the worst prognosis compared with node negative patients and patients with ITCs [25]. The clinical relevance of only finding ITCs and not micrometastasis therefore seems limited. Although no micrometastases were detected, the feasibility of the technique to accurately select the positive lymph nodes was demonstrated. Based on these results we advise that a study should be carried out with more patients to determine the clinical relevance of the magnetic technique.

An alternative technique for SLNM, using a near-infrared (NIR) fluorescent tracer and imaging system, has been described previously. This allows visualisation of tracer distribution from the injection sites to the sentinel nodes, and has been used *in vivo* and *ex vivo* in colorectal cancer [26-28]. Schaafsma et al and Hutteman et al. used an *ex vivo* approach and reported a sensitivity of 80% and 92 % for macrometastasis. However they did not perform ultrastaging on the sentinel nodes to detect the presence of occult tumour cells and therefore their results cannot be compared to ours. A limitation of this technique is that fluorescent tracers do not allow SLN detection after formalin fixation

due to migration to higher echelon nodes [27]. This makes the method less suitable for colorectal SLNM to improve the accuracy of staging in routine clinical practice.

The present study has demonstrated that the use of a magnetic tracer for *ex vivo* sentinel node selection is feasible. Rather than improving the accuracy of staging, the goal of an *in vivo* procedure is to allow for a limited resection of the increasing number of early stage tumours [29] or patient tailored determination of the extent of the lymphadenectomy [30]. Since the magnetic tracer Sienna+® is approved for *in vivo* use, and the magnetometer has a favourable detection depth of 2 to 3 centimetres, it could be used for this purpose , although no magnetometer for laparoscopic use is yet available. A limitation of the current study is the small number of patients, in particular the small number with occult metastasis. Larger studies are needed to validate the advantages of the magnetic technique over the blue dye technique.

In conclusion, this study has demonstrated that *ex vivo* magnetic SLNM is a feasible technique for use in routine clinical practice. The magnetic tracer can be detected in the LNs after formalin fixation, and the quantitative signal of the magnetometer can be used to objectively distinguish SLNs from higher echelon nodes. The magnetic technique has achieved an excellent identification rate, sensitivity and accuracy, and is superior to the blue dye technique. The technique can be used to improve staging accuracy of CRC patients in routine clinical practice.

ACKNOWLEDGEMENTS

This research is supported by the Dutch Technology Foundation STW, which is part of the Netherlands Organisation for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs.

Table 1 Patient and tumour characteristics of the included patients

Characteristic	**N**
Gender	
Male	*13*
Female	*14*
Age in years (mean ± SD, range)	73±6 (63-84)
Tumour size in cm (mean ± SD, range)	4.3±2.7 (1-11.5)
Tumour location	
Caecum	*5*
Ascending colon	*7*
Transverse colon	*1*
Descending colon	*1*
Sigmoid colon	*11*
Rectum	*3*
Depth of tumour invasion	
T1	*4*
T2	*6*
T3	*15*
T4	*3*

Table 2 Sentinel lymph node mapping procedure and ultrastaging results

Characteristic	**N**
Identification rate	
Magnetic	*27/28 (96%)*
Blue	*25/28 (89%)*
Number of dissected nodes (mean ± SD, range)	21.7±11.6 (7 - 54)
Magnetic	*7.4±4.1 (1-16)*
Blue	*8.2±6.6 (3-36)*
Total number of SLNs	120
Magnetic & Blue	*33*
Magnetic	*46*
Blue	*41*
Histology after ultrastaging	
Negative	*18*
Isolated tumour cells	*10*
Micrometastasis	*0*
False negatives after ultrastaging	
Magnetic	*0*
Blue	*1*

Figure 1 Selection of lymph nodes with uptake of magnetic tracer. A blue formalin-fixed lymph node is placed on the handheld probe (covered with a surgical glove) of the magnetometer with plastic tweezers. The numerical display of the system is used to quantify the uptake of magnetic tracer.

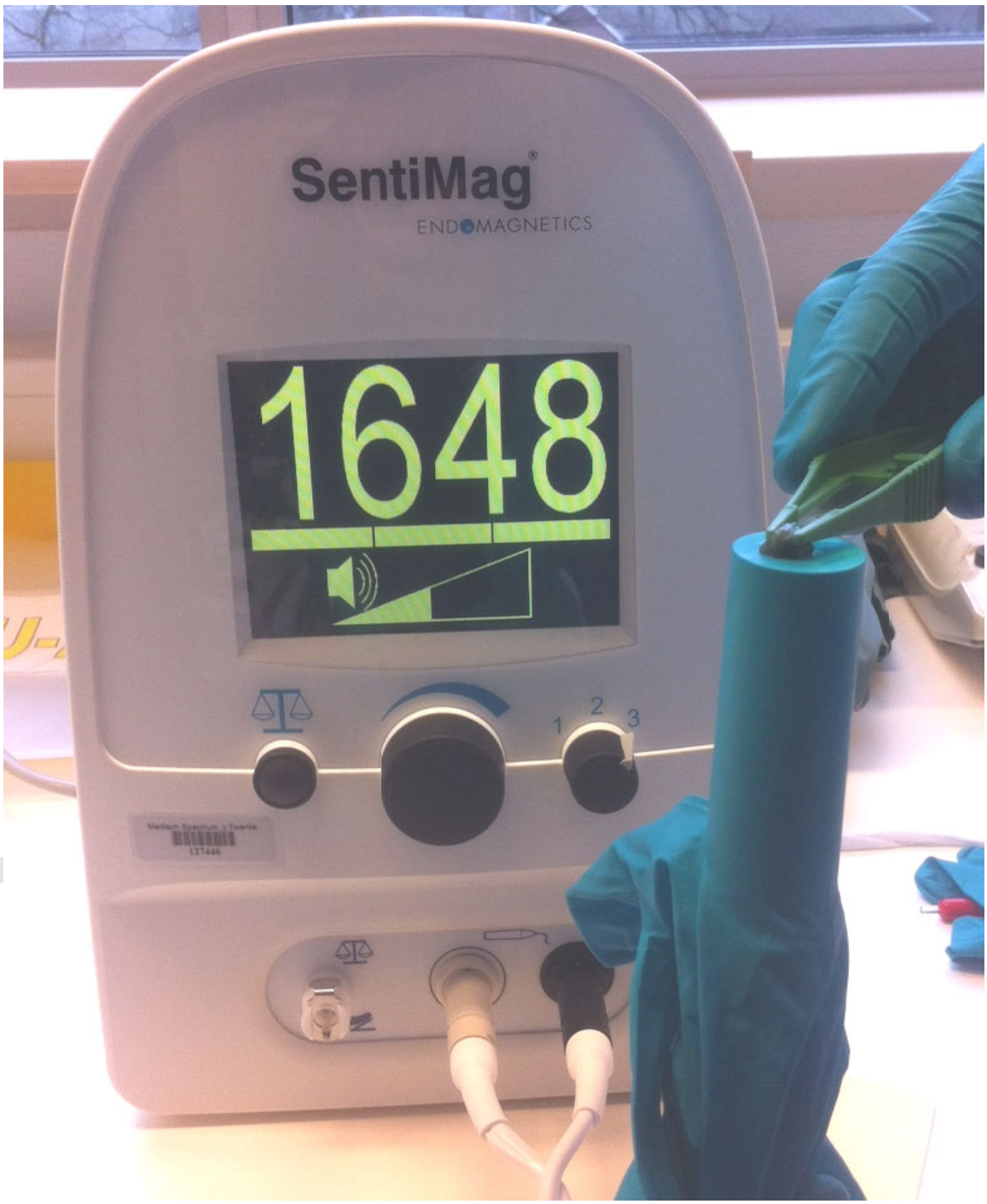
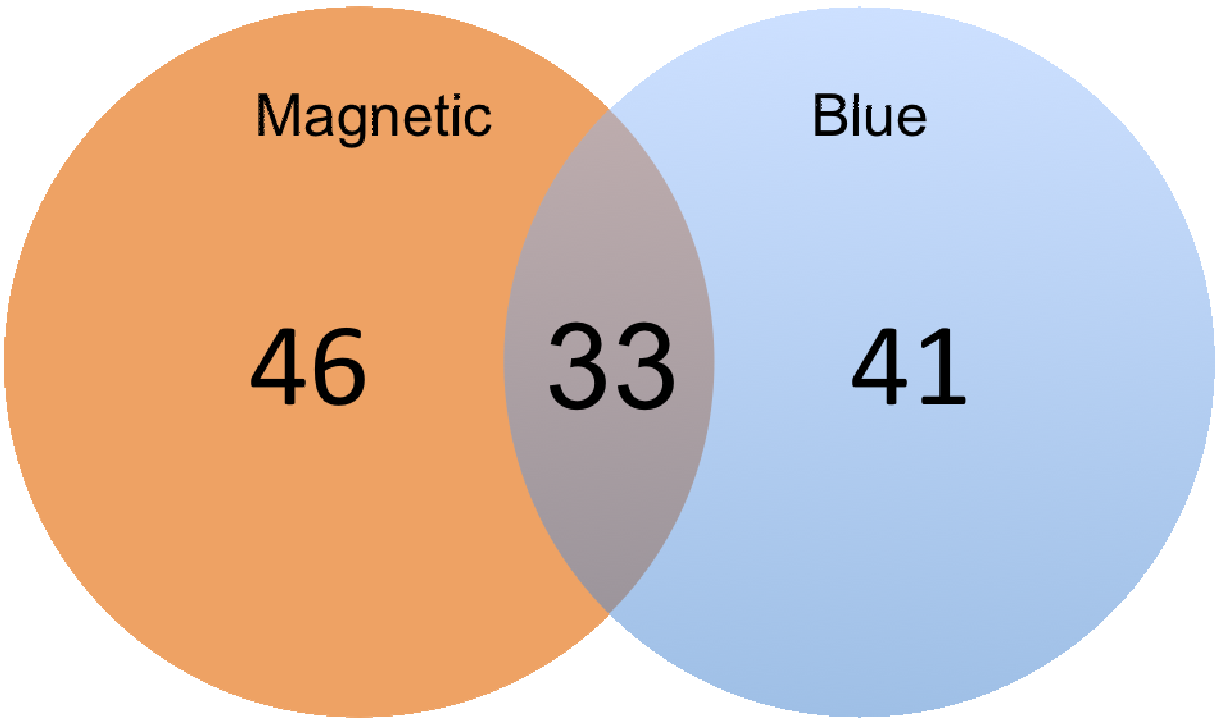


Figure 2 Number of magnetic and/or blue sentinel lymph nodes identified by the different techniques



REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359-86.
2. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Annals of surgical oncology. 2010;17(6):1471-4.
3. Bilchik AJ, DiNome M, Saha S, Turner RR, Wiese D, McCarter M, et al. Prospective multicenter trial of staging adequacy in colon cancer: preliminary results. Archives of surgery. 2006;141(6):527-33; discussion 33-4.
4. Figueredo A, Coombes ME, Mukherjee S. Adjuvant therapy for completely resected stage II colon cancer. Cochrane Database Syst Rev. 2008(3):CD005390.
5. van der Zaag ES, Kooij N, van de Vijver MJ, Bemelman WA, Peters HM, Buskens CJ. Diagnosing occult tumour cells and their predictive value in sentinel nodes of histologically negative patients with colorectal cancer. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2010;36(4):350-7.
6. Iddings D, Ahmad A, Elashoff D, Bilchik A. The prognostic effect of micrometastases in previously staged lymph node negative (N0) colorectal carcinoma: a meta-analysis. Annals of surgical oncology. 2006;13(11):1386-92.
7. Nicastri DG, Doucette JT, Godfrey TE, Hughes SJ. Is occult lymph node disease in colorectal cancer patients clinically significant? A review of the relevant literature. J Mol Diagn. 2007;9(5):563-71.
8. Quasar Collaborative G, Gray R, Barnwell J, McConkey C, Hills RK, Williams NS, et al. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. Lancet. 2007;370(9604):2020-9.
9. Tuech JJ, Pessaux P, Di Fiore F, Nitu V, Lefebure B, Colson A, et al. Sentinel node mapping in colon carcinoma: in-vivo versus ex-vivo approach. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2006;32(2):158-61.

10. Wood TF, Saha S, Morton DL, Tsioulias GJ, Rangel D, Hutchinson W, Jr., et al. Validation of lymphatic mapping in colorectal cancer: in vivo, ex vivo, and laparoscopic techniques. Annals of surgical oncology. 2001;8(2):150-7.
11. Thevarajah S, Huston TL, Simmons RM. A comparison of the adverse reactions associated with isosulfan blue versus methylene blue dye in sentinel lymph node biopsy for breast cancer. Am J Surg. 2005;189(2):236-9.
12. Bezu C, Coutant C, Salengro A, Darai E, Rouzier R, Uzan S. Anaphylactic response to blue dye during sentinel lymph node biopsy. Surgical oncology. 2011;20(1):e55-9.
13. Saha S, Dan AG, Berman B, Wiese D, Schochet E, Barber K, et al. Lymphazurin 1% versus 99mTc sulfur colloid for lymphatic mapping in colorectal tumors: a comparative analysis. Annals of surgical oncology. 2004;11(1):21-6.
14. Merrie AE, van Rij AM, Phillips LV, Rossaak JI, Yun K, McCall JL. Diagnostic use of the sentinel node in colon cancer. Diseases of the colon and rectum. 2001;44(3):410-7.
15. van der Pas MH, Meijer S, Hoekstra OS, Riphagen, II, de Vet HC, Knol DL, et al. Sentinel-lymph-node procedure in colon and rectal cancer: a systematic review and meta-analysis. The lancet oncology. 2011;12(6):540-50.
16. Visscher M, Pouw JJ, van Baarlen J, Klaase JM, Ten Haken B. Quantitative analysis of superparamagnetic contrast agent in sentinel lymph nodes using ex vivo vibrating sample magnetometry. IEEE transactions on bio-medical engineering. 2013;60(9):2594-602.
17. Douek M, Klaase J, Monypenny I, Kothari A, Zechmeister K, Brown D, et al. Sentinel node biopsy using a magnetic tracer versus standard technique: the SentiMAG Multicentre Trial. Annals of surgical oncology. 2014;21(4):1237-45.
18. Pouw JJ, Ahmed M, Anninga B, Schuurman K, Pinder SE, Van Hemelrijck M, et al. Comparison of three magnetic nanoparticle tracers for sentinel lymph node biopsy in an in vivo porcine model. International journal of nanomedicine. 2015;10:1235-43.
19. Viehl CT, Hamel CT, Marti WR, Guller U, Eisner L, Stammberger U, et al. Identification of sentinel lymph nodes in colon cancer depends on the amount of dye injected relative to tumor size. World J Surg. 2003;27(12):1285-90.
20. Anninga B, Ahmed M, Van Hemelrijck M, Pouw J, Westbroek D, Pinder S, et al. Magnetic sentinel lymph node biopsy and localization properties of a magnetic tracer in an in vivo porcine model. Breast cancer research and treatment. 2013;141(1):33-42.
21. Faerden AE, Sjo OH, Andersen SN, Hauglann B, Nazir N, Gravdehaug B, et al. Sentinel node mapping does not improve staging of lymph node metastasis in colonic cancer. Diseases of the colon and rectum. 2008;51(6):891-6.
22. ten Haken B, Visscher M, Pouw JJ, Klaase JM, Pankhurst QA, Galindo-Millan J, et al. Magnetic Detection of the Sentinel Lymph Node in Ex Vivo Tissue with Colorectal Cancer. Ifmbe Proc. 2010;28:447-9.
23. van der Zaag ES, Bouma WH, Tanis PJ, Ubbink DT, Bemelman WA, Buskens CJ. Systematic review of sentinel lymph node mapping procedure in colorectal cancer. Annals of surgical oncology. 2012;19(11):3449-59.
24. Smith FM, Coffey JC, Khasri NM, Walsh MF, Parfrey N, Gaffney E, et al. Sentinel nodes are identifiable in formalin-fixed specimens after surgeon-performed ex vivo sentinel lymph node mapping in colorectal cancer. Annals of surgical oncology. 2005;12(6):504-9.
25. Sloothaak DA, Sahami S, van der Zaag-Loonen HJ, van der Zaag ES, Tanis PJ, Bemelman WA, et al. The prognostic value of micrometastases and isolated tumour cells in histologically negative lymph nodes of patients with colorectal cancer: a systematic review and meta-analysis. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2014;40(3):263-9.
26. Hutteman M, Choi HS, Mieog JS, van der Vorst JR, Ashitate Y, Kuppen PJ, et al. Clinical translation of ex vivo sentinel lymph node mapping for colorectal cancer using invisible near-infrared fluorescence light. Annals of surgical oncology. 2011;18(4):1006-14.
27. Schaafsma BE, Verbeek FP, van der Vorst JR, Hutteman M, Kuppen PJ, Frangioni JV, et al. Ex vivo sentinel node mapping in colon cancer combining blue dye staining and fluorescence imaging. The Journal of surgical research. 2013;183(1):253-7.
28. Hirche C, Mohr Z, Kneif S, Doniga S, Murawa D, Strik M, et al. Ultrastaging of colon cancer by sentinel node biopsy using fluorescence navigation with indocyanine green. Int J Colorectal Dis. 2012;27(3):319-24.
29. Cahill RA, Perretta S, Leroy J, Dallemagne B, Marescaux J. Lymphatic mapping and sentinel node biopsy in the colonic mesentery by Natural Orifice Transluminal Endoscopic Surgery (NOTES). Annals of surgical oncology. 2008;15(10):2677-83.
30. Saha S, Johnston G, Korant A, Shaik M, Kanaan M, Johnston R, et al. Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation. Am J Surg. 2013;205(3):302-5; discussion 5-6.